

**WHAT IS CLAIMED IS:**

1. A method for calibrating detection of light from biological samples comprising:

providing a system adapted to excitation and detection of a plurality of spectrally distinguishable species, wherein the system comprises a plurality of filters;

providing a calibration plate comprising a plurality of wells, wherein each well comprises a sample with a spectrally distinguishable species;

detecting light from the spectrally distinguishable species for each well;

determining a correction factor for each spectrally distinguishable species for each well.

2. The method of claim 1, wherein each well comprises each spectrally distinguishable species.

3. The method of claim 1, wherein determining a correction factor further comprises determining a correction factor for each spectrally distinguishable species for each filter, for each well.

4. The method of claim 1, wherein each spectrally distinguishable species is associated with at least one filter of the plurality of filters.

5. The method of claim 1, wherein the plurality of spectrally distinguishable species comprises at least one dye chosen from FAM, SYBR Green, VIC, JOE, TAMRA, NED, CY-3, Texas Red, CY-5, and ROX.

6. The method of claim 1, further comprising normalizing the light detected from each well.

7. The method of claim 6, wherein the correction factor is determined prior to normalizing.

8. The method of claim 6, wherein the correction factor is determined after normalizing.

9. The method of claim 1, wherein the correction factor is determined from a plateau at the end of a run.

10. The method of claim 1, wherein the correction factor is determined from an unquenched dye at the beginning of a run.

11. The method of claim 1, wherein the correction factor is determined from log-log analysis of the run.

12. A method for calibrating detection of light from biological samples comprising:

providing a system adapted to excitation and detection of a plurality of spectrally distinguishable species, wherein the system comprises a plurality of filters;

providing a calibration plate comprising a plurality of wells, wherein each well comprises a sample with a spectrally distinguishable species;

detecting light from each filter for each well;

determining a correction factor for each filter.

13. The method of claim 12, wherein each well comprises each spectrally distinguishable species.

14. The method of claim 12, wherein determining a correction factor further comprises determining a correction factor for each spectrally distinguishable species for each filter, for each well.

15. The method of claim 12, wherein each spectrally distinguishable species is associated with at least one filter of the plurality of filters.

16. The method of claim 12, wherein the plurality of spectrally distinguishable species comprises at least one dye chosen from FAM, SYBR Green, VIC, JOE, TAMRA, NED, CY-3, Texas Red, CY-5, and ROX.

17. The method of claim 12, further comprising normalizing the light detected from each well.

18. The method of claim 17, wherein the correction factor is determined prior to normalizing.

19. The method of claim 17, wherein the correction factor is determined after normalizing.

20. The method of claim 1, wherein the correction factor is determined from a plateau at the end of a run.

21. The method of claim 1, wherein the correction factor is determined from an unquenched dye at the beginning of a run.

22. The method of claim 1, wherein the correction factor is determined from log-log analysis of the run.

23. A system for detection of light from biological samples, the system comprising:

a detector;

a plurality of filters;

a plurality of spectrally distinguishable species; and

a plurality of wells,

wherein the detector is adapted to determine a correction factor for each spectrally distinguishable species, for each filter, for each well.

24. The system of claim 23, further comprising a calibration plate.

25. The system of claim 24, wherein the calibration plate provides light to the detector for one spectrally distinguishable species calibration.

26. The system of claim 24, wherein the calibration plate provides light to the detector for each spectrally distinguishable species calibration.

27. A computer-readable software comprising code adapted to calibrate detection of light from a biological sample, the code providing commands comprising:

determining a correction factor for each of a plurality of spectrally distinguishable species in the biological sample, wherein determining comprises (1) generating a set of simultaneous equations for image data detected from each of the spectrally distinguishable species, and (2) solving the set of simultaneous equations for a contribution to the correction factor of each of the spectrally distinguishable species; and

normalizing the image data detected from the biological sample.

28. The computer-readable software of claim 27, wherein determining further comprises determining a correction factor for each spectrally distinguishable species, for each of a plurality of wells adapted to house the biological sample.

29. The computer-readable software of claim 28, wherein determining further comprises determining a correction factor for each spectrally distinguishable species, for each of a plurality of filters corresponding to the plurality of spectrally distinguishable species, for each of a plurality of wells adapted to house the biological sample.

30. A system for detection of light from biological samples, the system comprising:

means for detecting the light from the biological samples;

means for filtering the light from a plurality of spectrally distinguishable species, wherein the filtering is adapted to each spectrally distinguishable species;

means of containing an array of biological samples, wherein the light from each sample provides a different angle to the means for detecting; and

means for determining a correction factor for each spectrally distinguishable

species, wherein the correction factor has components for filtering and containing.

31. A calibration plate for detection of light from biological samples, the plate comprising:

a plurality of wells;

a plurality of samples; and

a spectrally distinguishable species,

wherein each well comprises a sample, wherein each sample comprises a spectrally distinguishable species, wherein each well is adapted to provide a correction factor for a plurality of filters.

32. The calibration plate of claim 31, wherein each sample comprises a plurality of spectrally distinguishable species.

33. A calibration plate for detection of light from biological samples, the plate comprising:

a substrate comprising a plurality of locations, wherein each location is adapted to provide light representing a plurality of spectrally distinguishable species to determine a correction factor for a plurality of filters for each location.

34. The calibration plate of claim 33, wherein each location is adapted to reflect light representing the plurality of spectrally distinguishable species.

35. The calibration plate of claim 34, wherein the substrate comprises two thin solid plates to reflect the light.

36. The calibration plate of claim 33, wherein the substrate is coated.